C-Terminal 5.0 Protein Sequencer Methods

Technical Note

HP 241 Protein Sequencer (N+C)

The C-terminal 5.0 sequencer methods implement the thiohydantoin degradation chemistry (coupling and cleavage) on the Hewlett-Packard column-based protein sequencer. Reagents R1, R2A, R4, and the PTH-Std are specific for N-terminal sequencing and are not utilized for the C-terminal 5.0 sequencer methods.

These methods are suitable for C-terminal sequence analysis of most protein samples in the low nanomole to 50 pmole range which have been applied onto a Hewlett-Packard Zitex strip. Once dry the strip is loaded into an empty RP/SAX biphasic column. No column preparation is required with this method.

The methods are:

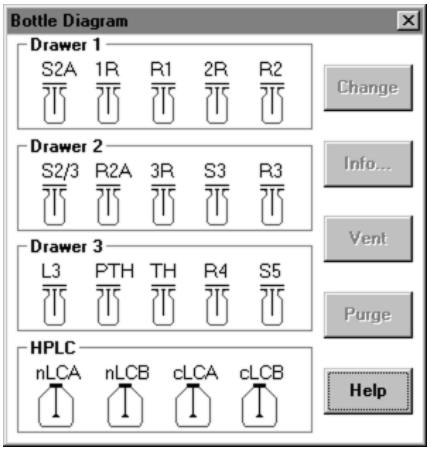
- C-Terminal 5.0
- C-Terminal 5.0 (Cycle 1)
- C-Terminal Flask 5.0
- TH Std 5.0

The column and flask methods control all of the derivatization and cleavage reactions for the thiohydantoin chemistry.

The sequence program controls all of the column and flask methods, as well as any cycle exception methods. The C-terminal 5.0 sequencer method uses a cycle 1 column exception method.

The cycle 1 method provides a longer initial drying time and a pause to provide time for proper HPLC column equilibration after switching from N-terminal sequencing. There is no specialized cycle 1 method for the flask.

The TH Std 5.0 method delivers 50 pmol/100 µl TH-amino acid standard solution to the on-line HPLC allowing the quantitation and identification of sequenced amino acids. One may choose to run a TH-standard by using TH-std 5.0 when scheduling a sample.



S2A	Neat ethyl acetate
1R	Diphenylphosphorylisothiocyanate (DPP-ITC) in toluene/heptane (23:27:50)
R1	Phenylisothiocyanate (PITC) in heptane (3:97)
2R	Pyridine in ethylacetate (2:98)
R2	Diisopropylethylamine (DIEA) in 1-propanol/water (1:6:3)
S2/3	Acetonitrile/toluene (23:77)
R2A	Ocylamine in heptane (3:97)
3R	Potassium trimethylsilanolate (0.1M) in methanol/t-butanol (50:50)
S3	Acetonitrile/toluene (15:85)
R3	Neat trifluoroacetic acid (TFA)
L3	Acetic acid in methanol/water (1:74:25)
PTH	Mixture of PTH amino acids (10 pmol/100 µl) in acetonitrile with DPTU as a marker
TH	Mixture of TH amino acids (50 pmol/100 µl) in acetonitrile
R4	Trifluoroacetic acid (TFA) in water (1:3)
S5	Phosphate buffer (pH 2.9), 0.2% ion pairing reagent

Method Details

The steps for the C-terminal 5.0 methods are described below. The steps indicate the ranges in volume that are appropriate for the various reagents/solvent deliveries. All sequencer methods are accessed by choosing the top menu item Edit/Method in the Protein Sequencer window. The sequencer column configuration used with the C-terminal 5.0 methods consists of an empty reverse-phase (RP) sample column (top) mated with an empty strong anion exchange (SAX) column (bottom).

Method (Column):
C-Terminal 5.0

Total time: 75.2 min

All metering steps deliver the specified reagent or solvent to waste.

Steps 3, 25, 47, 72: The volume of R3 delivered during this step should be just enough to wet the membrane.

Steps 7, 13, 19, 22, 29, 35, 41, 44, 51, 57, 63, 66, 69,76, 79, 82, 88, 91, 109, 112: The delivery of S2/3 and S3 should completely wet the Zitex membrane. The solvent deliveries are held against a closed valve, enabling 1/2 to 3/4 of the membrane-containing column compartment (bottom) to remain filled with solvent for the duration of the step.

Steps 10, 32, 54: The delivery of 1R should completely wet the membrane, but not flood it. The initial delivered volume (about 1/2 the volume of the column) should blow through the column wetting the Zitex strip. The 1R reagent delivery is then held against a closed valve, enabling only a small volume of 1R to sit at the bottom of the column (approximately 1 slit distance as measured by the slits on the column wings).

Step	Description	Primary Time	Temperature
1:	Activation: Dry column DOWN	60.0	60
2:	Activation: Meter R3	7.0	60
3:	Activation: Deliver R3 DOWN	30.0	60
4:	Wash: Flush RV2 with S3	5.0	80
5:	Activation: Dry column DOWN	50.0	80
6:	Wash: Meter S2/3	9.0	80
7:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
8:	Wash: Dry column DOWN	5.0	80
9:	Couple: Meter 1R	5.0	80
10:	Couple: Deliver 1R UP	30.0	80
11:	Couple: Dry column DOWN	10.0	80
12:	Wash: Meter S2/3 and S2A	9.0	80
13:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
14:	Wash: Dry column DOWN	5.0	80
15:	Couple: Meter 2R	5.0	80
16:	Couple: Deliver 2R UP	10.0	80
17:	Couple: Dry column DOWN	5.0	80
18:	Wash: Meter S2/3 and S2A	9.0	80
19:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
20:	Wash: Dry column DOWN	5.0	80
21:	Wash: Meter S2/3	9.0	80
22:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
23:	Wash: Dry column DOWN	20.0	80
24:	Activation: Meter R3	7.0	60
25:	Activation: Deliver R3 DOWN	30.0	60
26:	Wash: Flush RV2 with S3	5.0	80
27:	Activation: Dry column DOWN	50.0	80
28:	Wash: Meter S2/3	9.0	80
29:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
30:	Wash: Dry column DOWN	5.0	80
31:	Couple: Meter 1R	5.0	80
32:	Couple: Deliver 1R UP	30.0	80
33:	Couple: Dry column DOWN	10.0	80
34:	Wash: Meter S2/3 and S2A	9.0	80
35:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
36:	Wash: Dry column DOWN	5.0	80
37:	Couple: Meter 2R	5.0	80
38:	Couple: Deliver 2R UP	10.0	80
39:	Couple: Dry column DOWN	5.0	80
40:	Wash: Meter S2/3 and S2A	9.0	80
41:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
42:	Wash: Dry column DOWN	5.0	80
43:	Wash: Meter S2/3	9.0	80
44:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
45:	Wash: Dry column DOWN	20.0	80
46:	Activation: Meter R3	7.0	60
47:	Activation: Deliver R3 DOWN	30.0	60
48:	Wash: Flush RV2 with S3	5.0	80
49:	Activation: Dry column DOWN	50.0	80
50:	Wash: Meter S2/3	9.0	80
51:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
52:	Wash: Dry column DOWN	5.0	80

Steps 16, 38, 60: The delivery of 2R should completely wet the membrane. The initial delivered volume (about 1/2 the volume of the column) should blow through the column wetting the Zitex strip. The 2R reagent delivery is then held against a closed valve, enabling only a small volume of 2R to sit at the bottom of the column (approximately 1 slit distance as measured by the slits on the column wings).

Steps 12, 18, 34, 40, 56, 62: "Meter S2/3 and S2A". The S2A flushes the delivery lines and should not wet the column. The S2/3 is involved in the column wash and is sent to the column.

Steps 85, 106: The volume of R2 delivered during this step should not wet the membrane.

Steps 86, 107: During the high pressure dry step, the volume of R2 delivered should be just enough to wet the Zitex membrane. Note: The R2 reagent does not actually "wet" the Zitex membrane like other reagents and solvents. The membrane will not change its appearance in the presence of R2.

Step 94: After Step 94, the volume of L3 delivered to the flask should be 50 µl (+/- 5 µl)

Steps 96, 101: The delivery of 3R should completely wet the membrane, but not flood it. The 3R reagent delivery is then held against a closed valve, enabling only a small volume of 3R to sit at the bottom of the column (approximately 1 slit distance as measured by the slits on the column wings).

Step	Description	Primary Time	Temperature
53:	Couple: Meter 1R	5.0	80
54:	Couple: Deliver 1R UP	30.0	80
55:	Couple: Dry column DOWN	10.0	80
56:	Wash: Meter S2/3 and S2A	9.0	80
57:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
58:	Wash: Dry column DOWN	5.0	80
59:	Couple: Meter 2R	5.0	80
60:	Couple: Deliver 2R UP	10.0	80
61:	Couple: Dry column DOWN	5.0	80
62:	Wash: Meter S2/3 and S2A	9.0	80
63:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
64:	Wash: Dry column DOWN	5.0	80
65:	Wash: Meter S2/3	9.0	80
66:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
67:	Wash: Dry column DOWN	5.0	80
68:	Wash: Meter S3	9.0	80
69:	Wash: Deliver S3 DOWN (closed)	15.0	80
70:	Wash: Dry column DOWN	30.0	80
71:	Cleave: Meter R3	7.0	80
72:	Cleave: Deliver R3 DOWN	400.0	80
73:	Wash: Flush RV2 with S3	5.0	80
74:	Cleave: Dry column DOWN	160.0	80
75:	!Extract: Meter S3	9.0	80
76:	!Extract: Deliver S3 DOWN to flask	15.0	80
77:	!Extract: Dry column UP	10.0	60
78:	!Cleave: Meter S2/3	10.0	50
79:	!Cleave: Deliver S2/3 DOWN	30.0	50
80:	!Cleave: Dry column	10.0	50
81:	!Cleave: Meter S2/3	10.0	50
82:	!Cleave: Deliver S2/3 DOWN	30.0	50
83:	!Cleave: Dry column	30.0	50
84:	!Cleave: Meter R2	10.5	55
85:	!Cleave: Deliver R2 DOWN	120.0	55
86:	!Cleave: Dry column	60.0	50
87:	!Cleave: Meter S2/ 3	10.0	50
88:	!Cleave: Deliver S2/3 DOWN	30.0	50
89:	!Cleave: Dry column	30.0	50
90:	!Cleave: Meter S2/3	10.0	50
91:	!Cleave: Deliver S2/3 DOWN	30.0	50
92:	!Cleave: Dry column	30.0	50
93:	!Cleave: Meter L3	7.0	40
94:	!Cleave: Deliver L3 to transfer flask	25.0	40
95:	!Cleave: Meter 3R	9.0	50
96:	!Cleave: Cleave and extract to flask	30.0	50
97:	!Extract: Meter S3	10.0	50
98:	!Extract: Extract to flask UP	10.0	50
99:	!Cleave: Dry column UP	30.0	50
100:	!Cleave: Meter 3R	9.0	50
101:	!Cleave: Cleave and extract to flask	30.0	50
102:	!Extract: Meter S3	10.0	50
103:	!Extract: Extract to flask UP	10.0	50
104:	!Cleave: Dry column UP	20.0	55
105:	Wash: Meter R2	10.5	55
106:	Wash: Deliver R2 DOWN	120.0	55
107:	Wash: Dry column DOWN	60.0	60
108:	Wash: Meter S2/3	9.0	60
109:	Wash: Deliver S2/3 DOWN (closed)	15.0	60
110:	Wash: Dry column DOWN	15.0	60
111:	Wash: Meter S2/3	9.0	60
112:	Wash: Deliver S2/3 DOWN (closed)	15.0	60
113:	Wash: Dry column DOWN	20.0	60

! indicates system step

Method (Column): C-Terminal 5.0 (Cycle 1)

Total time: 88.7 min

Step 1: Cycle 1 begins with an extended dry to remove any residual solvent from the sample loading process. There is also a pause to provide time for proper HPLC column equilibration after switching from N-terminal sequencing

Steps 3, 25, 47, 72: The volume of R3 delivered during this step should be just enough to wet the membrane.

Steps 7, 13, 19, 22, 29, 35, 41, 44, 51, 57, 63, 66, 69,76, 79, 82, 88, 91, 109, 112: The delivery of S2/3 and S3 should completely wet the Zitex membrane. The solvent deliveries are held against a closed valve, enabling 1/2 to 3/4 of the membrane-containing column compartment (bottom) to remain filled with solvent for the duration of the step.

Steps 10, 32, 54: The delivery of 1R should completely wet the membrane, but not flood it. The initial delivered volume (about 1/2 the volume of the column) should blow through the column wetting the Zitex strip. The 1R reagent delivery is then held against a closed valve, enabling only a small volume of 1R to sit at the bottom of the column (approximately 1 slit distance as measured by the slits on the column wings).

Steps 16, 38, 60: The delivery of 2R should completely wet the membrane. The initial delivered volume (about 1/2 the volume of the column) should blow through the column wetting the Zitex strip. The 2R reagent delivery is then held against a closed valve, enabling only a small volume of 2R to sit at the bottom of the column (approximately 1 slit distance as measured by the slits on the column wings).

Step	Description	Primary Time	Temperature
1:	Activation: Dry column DOWN and wait	120.0	60
2:	Activation: Meter R3	7.0	60
3:	Activation: Deliver R3 DOWN	30.0	60
4:	Wash: Flush RV2 with S3	5.0	80
5:	Activation: Dry column DOWN	50.0	80
6:	Wash: Meter S2/3	9.0	80
7:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
8:	Wash: Dry column DOWN	5.0	80
9:	Couple: Meter 1R	5.0	80
10:	Couple: Deliver 1R UP	30.0	80
11:	Couple: Dry column DOWN	10.0	80
12:	Wash: Meter S2/3 and S2A	9.0	80
13:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
14:	Wash: Dry column DOWN	5.0	80
15:	Couple: Meter 2R	5.0	80
16:	Couple: Deliver 2R UP	10.0	80
17:	Couple: Dry column DOWN	5.0	80
18:	Wash: Meter S2/3 and S2A	9.0	80
19:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
20:	Wash: Dry column DOWN	5.0	80
21:	Wash: Meter S2/3	9.0	80
22:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
23:	Wash: Dry column DOWN	20.0	80
24:	Activation: Meter R3	7.0	60
25:	Activation: Deliver R3 DOWN	30.0	60
26:	Wash: Flush RV2 with S3	5.0	80
27:	Activation: Dry column DOWN	50.0	80
28:	Wash: Meter S2/3	9.0	80
29:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
30:	Wash: Dry column DOWN	5.0	80
31:	Couple: Meter 1R	5.0	80
32:	Couple: Deliver 1R UP	30.0	80
33:	Couple: Dry column DOWN	10.0	80
34:	Wash: Meter S2/3 and S2A	9.0	80
35:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
36:	Wash: Dry column DOWN	5.0	80
37:	Couple: Meter 2R	5.0	80
38:	Couple: Deliver 2R UP	10.0	80
39:	Couple: Dry column DOWN	5.0	80
40:	Wash: Meter S2/3 and S2A	9.0	80
41:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
42:	Wash: Dry column DOWN	5.0	80
43:	Wash: Meter S2/3	9.0	80
44:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
45:	Wash: Dry column DOWN	20.0	80
46:	Activation: Meter R3	7.0	60
47:	Activation: Deliver R3 DOWN	30.0	60
48:	Wash: Flush RV2 with S3	5.0	80
49:	Activation: Dry column DOWN	50.0	80
50:	Wash: Meter S2/3	9.0	80
51:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
52:	Wash: Dry column DOWN	5.0	80
53:	Couple: Meter 1R	5.0	80
54:	Couple: Deliver 1R UP	30.0	80
55:	Couple: Dry column DOWN	10.0	80
56:	Wash: Meter S2/3 and S2A	9.0	80

Steps 12, 18, 34, 40, 56, 62: "Meter S2/3 and S2A". The S2A flushes the delivery lines and should not wet the column. The S2/3 is involved in the column wash and is sent to the column.

Steps 85, 106: The volume of R2 delivered during this step should not wet the membrane.

Steps 86, 107: During the high pressure dry step, the volume of R2 delivered should be just enough to wet the Zitex membrane. Note: The R2 reagent does not actually "wet" the Zitex membrane like other reagents and solvents. The membrane will not change its appearance in the presence of R2.

Step 94: After Step 94, the volume of L3 delivered to the flask should be 50 µl (+/- 5 µl)

Steps 96, 101: The delivery of 3R should completely wet the membrane, but not flood it. The 3R reagent delivery is then held against a closed valve, enabling only a small volume of 3R to sit at the bottom of the column (approximately 1 slit distance as measured by the slits on the column wings).

Step	Description	Primary Time	Temperature
57:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
58:	Wash: Dry column DOWN	5.0	80
59:	Couple: Meter 2R	5.0	80
60:	Couple: Deliver 2R UP	10.0	80
61:	Couple: Dry column DOWN	5.0	80
62:	Wash: Meter S2/3 and S2A	9.0	80
63:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
64:	Wash: Dry column DOWN	5.0	80
65:	Wash: Meter S2/3	9.0	80
66:	Wash: Deliver S2/3 DOWN (closed)	15.0	80
67:	Wash: Dry column DOWN	5.0	80
68:	Wash: Meter S3	9.0	80
69:	Wash: Deliver S3 DOWN (closed)	15.0	80
70:	Wash: Dry column DOWN	30.0	80
71:	Cleave: Meter R3	7.0	80
72:	Cleave: Deliver R3 DOWN	400.0	80
73:	Wash: Flush RV2 with S3	5.0	80
74:	Cleave: Dry column DOWN	160.0	80
75:	!Extract: Meter S3	9.0	80
76:	!Extract: Deliver S3 DOWN to flask	15.0	80
77:	!Extract: Dry column UP	10.0	60
78:	!Cleave: Meter S2/3	10.0	50
79:	!Cleave: Deliver S2/3 DOWN	30.0	50
80:	!Cleave: Dry column	10.0	50
81:	!Cleave: Meter S2/3	10.0	50
82:	!Cleave: Deliver S2/3 DOWN	30.0	50
83:	!Cleave: Dry column	30.0	50
84:	!Cleave: Meter R2	10.5	55
85:	!Cleave: Deliver R2 DOWN	120.0	55
86:	!Cleave: Dry column	60.0	50
87:	!Cleave: Meter S2/3	10.0	50
88:	!Cleave: Deliver S2/3 DOWN	30.0	50
89:	!Cleave: Dry column	30.0	50
90:	!Cleave: Meter S2/3	10.0	50
91:	!Cleave: Deliver S2/3 DOWN	30.0	50
92:	!Cleave: Dry column	30.0	50
93:	!Cleave: Meter L3	7.0	40
94:	!Cleave: Deliver L3 to transfer flask	25.0	40
95:	!Cleave: Meter 3R	9.0	50
96:	!Cleave: Cleave and extract to flask	30.0	50
97:	!Extract: Meter S3	10.0	50
98:	!Extract: Extract to flask UP	10.0	50
99:	!Cleave: Dry column UP	30.0	50
100:	!Cleave: Meter 3R	9.0	50
101:	!Cleave: Cleave and extract to flask	30.0	50
102:	!Extract: Meter S3	10.0	50
103:	!Extract: Extract to flask UP	10.0	50
104:	!Cleave: Dry column UP	20.0	55
105:	Wash: Meter R2	10.5	55
106:	Wash: Deliver R2 DOWN	120.0	55
107:	Wash: Dry column DOWN	60.0	60
108:	Wash: Meter S2/3	9.0	60
109:	Wash: Deliver S2/3 DOWN (closed)	15.0	60
110:	Wash: Dry column DOWN	15.0	60
111:	Wash: Meter S2/3	9.0	60
112:	Wash: Deliver S2/3 DOWN (closed)	15.0	60
113:	Wash: Dry column DOWN	20.0	60

! indicates system step

Method (Flask): C-Terminal Flask 5.0

Total time: 30.5 min

Step 1: During Step 1, the liquid in the flask is slowly and completely dried

Step 2: By the end of Step 2, 70 µl (+/- 5 µl) of L3 will have been delivered to the flask

Step 3: By the end of Step 3, the flask will have been dry for 75-100 seconds

Step 5: After Step 5, the volume of S5 in the flask should be 75-80 µl.

TEST Delivery protocol:
To check the delivery volume of S5, copy Steps 4 and 5 to the clipboard and run from the clipboard. At the end of Step 5, remove the flask from the heating chamber. Carefully unscrew the flask cap and measure the delivered volume using a syringe. Be careful that none of the delivered volume is retained on the tube sides or top of the flask.

Step 8: Before injection the injector loop should be filled, leaving the solvent visible in both the inlet and outlet lines of the injector loop. The time for Step 8 may have to be adjusted in order to optimize the delivery.

Step	Description	Primary Time	Temperature
1:	Transfer: Evaporate	240.0	50
2:	Transfer: Deliver L3	10.0	50
3:	Transfer: Evaporate dry L3	140.0	50
4:	Transfer: Meter S5	12.0	50
5:	Transfer: Deliver S5	14.0	50
6:	Transfer: Mix and equilibrate	10.0	50
7:	Transfer: Solubilize	15.0	50
8:	Transfer: Fill loop and inject	2.0	50
9:	Transfer: Empty flask	60.0	50
10:	Transfer: Flush with L3	60.0	50
11:	Transfer: Mix	20.0	50
12:	Transfer: Empty flask	30.0	50
13:	Transfer: Deliver L3 to V7	15.0	50
14:	Transfer: Flush with L3	60.0	50
15:	Transfer: Mix	20.0	50
16:	Transfer: Empty flask	30.0	50
17:	Transfer: Dry waste line	100.0	50
18:	Transfer: Dry vent line	100.0	50

Method (Flask): TH Std 5.0
Total time: 39.8 min

Fifty picomoles of TH standard are delivered to the flask in this method.

Step 1: Cycle 1 begins with a pause to provide time for proper HPLC column equilibration after switching from N-terminal sequencing.

Step 7: Step 7 should add 100 µl (+/- 2 µl) of TH-standard to the dry flask

TEST Delivery protocol:
To check the delivery volume of the TH-standards, copy the steps in the TH Std Test method to the clipboard and run from the clipboard. At the end of test program, remove the flask from the heating chamber. Carefully unscrew the flask cap and measure the delivered volume using a syringe. Be careful that none of the delivered volume is retained on the tube sides or top of the flask

Step 10: After Step 10, the volume of S5 in the flask should be 75-80 µl.

TEST Delivery protocol:
To check the delivery volume of S5, copy Steps 9 and 10 to the clipboard and run from the clipboard. At the end of Step 10, remove the flask from the heating chamber. Carefully unscrew the flask cap and measure the delivered volume using a syringe. Be careful that some of the delivered volume is not retained on the tube sides or top of the flask.

Step 13: Before injection the injector loop should be filled, leaving the solvent visible in both the inlet and outlet lines of the injector loop. The time for Step 13 may have to be adjusted in order to optimize the delivery.

Step	Description	Primary Time	Temperature
1:	Transfer: Empty flask	150.0	50
2:	Transfer: Meter L3	60.0	50
3:	Transfer: Deliver L3	25.0	50
4:	Transfer: Empty flask	60.0	50
5:	Transfer: Dry waste line	180.0	50
6:	Transfer: Dry vent line	180.0	50
7:	Transfer: Deliver TH standard	7.9	50
8:	Transfer: Evaporate	300.0	50
9:	Transfer: Meter S5	12.0	50
10:	Transfer: Deliver S5	14.0	50
11:	Transfer: Mix and equilibrate	10.0	50
12:	Transfer: Solubilize	15.0	50
13:	Transfer: Fill loop and inject	2.0	50
14:	Transfer: Empty flask	30.0	50
15:	Transfer: Meter L3	60.0	50
16:	Transfer: Deliver L3	25.0	50
17:	Transfer: Empty flask	60.0	50
18:	Transfer: Meter L3	60.0	50
19:	Transfer: Deliver L3	25.0	50
20:	Transfer: Empty flask	60.0	50
21:	Transfer: Dry waste line	120.0	50
22:	Transfer: Dry vent line	120.0	50

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